Retention and bioaccessibility of β-carotene in cassava (*Manihot esculanta*, Crantz) are affected by style of cooking

Sagar K. Thakkar[1] and Mark L. Failla[1, 2]

[1]Interdisciplinary PhD Program in Nutrition, The Ohio State University and [2]Department of Human Nutrition, The Ohio State University

INTRODUCTION

- Eradication of vitamin A deficiency still remains to be a global public health challenge (1)
- Along with supplementation, fortification and dietary diversification, biofortification has been proposed as a complementary and a sustainable approach in combat against vitamin A deficiency (2)
- Cassava (*Manihot esculanta*, Crantz) is consumed by more than 10% of population worldwide on daily basis and is one of the crops targeted for biofortification of pro-vitamin A carotenoids (3, 4)
- We previously demonstrated that the quantity of β-carotene (BC) partitioning in mixed micelles during simulated small intestinal digestion, i.e., the bioaccessibility of boiled cassava is highly correlated with BC content of different cultivars (5)
- However, cassava is also consumed after traditional preparation that requires chopping, grating, fermentation, boiling, and roasting
- Preparation of gari requires fermentation of grated cassava for three days in dark, followed by dehydration and roasting to produce granules
- *Fufu* is prepared by five day fermentation of chopped cassava in water followed by mashing, sieving and boiling

SPECIFIC AIM

The objective of the current study was to compare the retention and in vitro bioaccessibility of pro-vitamin A carotenoids in gari (roasted cassava granules) and fufu (fermented, cooked cassava paste) and boiled cassava for three cultivars that were selected for their relatively high BC content

ABSTRACT

We previously demonstrated that the bioaccessibility of β-carotene (BC) in boiled cassava from various cultivars is highly correlated with BC content. However, cassava is also prepared by fermentation followed by either boiling (*fufu*) or roasting (*gari*). The potential impact of such processing on retention and bioaccessibility of BC was investigated. We first compared retention of BC in boiled cassava, *gari*, and *fufu* each prepared from the roots of three different cultivars. BC content in unprocessed cultivars was 6-8 µg/g wet weight and *cis* isomers accounting for approximately one-third of total BC. Apparent retention of BC was approximately 90% for boiled cassava and *fufu*. In contrast, roasting fermented cassava at 195°C for 20 min to prepare *gari* decreased BC content by 90%. Retention was increased to 63% when roasting temperature was reduced to 165°C for 10 min. Processing also was associated with a decline in all *trans* -BC and concomitant increase in 13-*cis* BC. The efficiency of micellarization of all *trans* and *cis* isomers of BC during simulated digestion was 25-30% for both boiled cassava and *gari* and independent of cultivar, but only 12-15% for *fufu*. These differences in retention and bioaccessibility of BC from cassava processed in traditional styles suggest that *gari* and *fufu* likely provide fewer retinol activity equivalents than an equivalent caloric intake of boiled cassava.

MATERIALS AND METHODS

1.1 CASSAVA VARIETIES

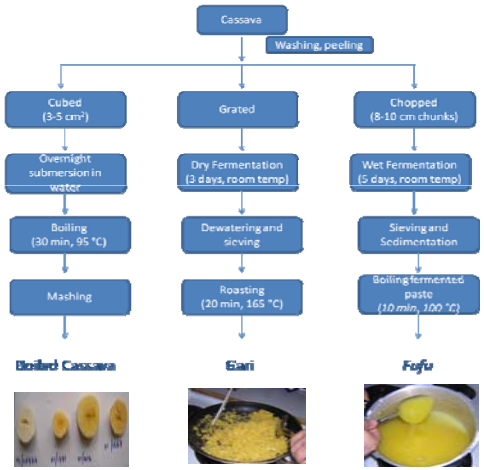
Varieties of cassava were planted at the beginning of the rainy season in 2006 and grown under rain-fed conditions in a randomized complete block design with two replications at the research farm of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Fertilizer and herbicides were not applied during growth, and hand weeding was done as needed. Roots used for this study were from three cultivars previously determined to contain relatively high amounts of total BC (5). Roots were harvested in summer 2007, washed, peeled, dipped in liquid nitrogen, and wrapped in aluminum foil before shipping on dry ice to The Ohio State University, Columbus, OH.

1.2 CALORIE CONTENT OF COOKED CASSAVA

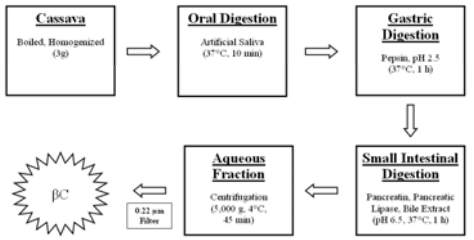
Cooked varieties of cassava (cultivar TMS 01/1371) were dried to constant mass at 105 °C. Pellets of 0.5 g dried mass were prepared for combustion in an oxygen bomb calorimeter (model 1281, Parr Instrument Company, Moline, IL). Energy content was 3.8 ± 0.1, 3.8 ± 0.1, and 4.0 ± 0.2 kcal/g dry weight for boiled cassava, gari, and *fufu*, respectively (n = 3).

MATERIALS AND METHODS

1.3 PREPARATION OF CASSAVA



1.4 IN VITRO DIGESTION (5)



1.5 EXTRACTION OF CAROTENOIDS FROM CASSAVA TUBERS, DIGESTA AND MICELLE FRACTION

The extraction of carotenoids from cassava was adopted from Kimura *et al.* (6) Generally, 15 g of thawed cassava was homogenized in 50 mL cold (4°C) THF:MeOH (1:1) for 90 sec. Sudan I (50 µg) in ethanol was added as recovery standard prior to homogenization. The homogenized extract was then filtered through a 55 mm Büchner funnel filter paper. Extraction of the pulp was repeated until residue and filtrate were colorless. Filtrates were pooled and transferred to a 500 mL separatory funnel with Teflon stop-cock containing 30 mL petroleum ether (PE). DI water (300 mL; 4°C) was then added slowly along the wall of the funnel to wash organic layer and enhance phase separation. After 10 min, aqueous phase was discarded and PE phase was washed 3 times with 200 mL cold DI water. PE containing carotenoids was then collected in 50 mL volumetric flask by passing it over 20 g of anhydrous sodium sulfate to remove residual water and through glass wool disc. Sodium sulfate and glass wool disc was then washed with 10 mL of petroleum ether to remove residual carotenoids. The solution was diluted to 50 mL with PE and 1 mL aliquots were transferred to HPLC vials for carotenoid analysis. Carotenoids from digesta and micelle fraction were extracted as described by Thakkar *et al.* (5)

1.6 HPLC ANALYSES

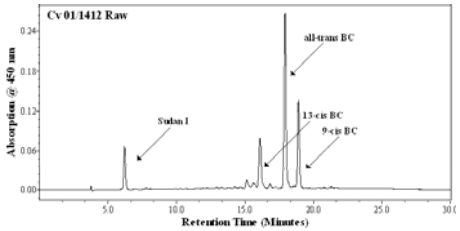
A Waters 2695 separation module with automated injector and a Waters 2996 photodiode array detector (PDA) were used to quantify carotenoids. Compounds in eluate were detected and quantified from their absorbance at 450 nm. Separation of carotenoids was achieved at 25°C using a Waters YMCTM Carotenoid S-5 C30 reversed-phase column (4.6 mm × 250 mm; particle size - 5 µm). The mobile phase consisted of methanol and ammonium acetate 1 mol/L (98:2) [Solvent A] and MTBE [Solvent B] with a gradient flow rate of 0.8 mL/min to 1.0 mL/min. The injection volume was 20 µL and carotenoids were eluted using the following solvent gradient: 0-10 min, 80% A; 11-20 min, 60% A; 21-25 min, 40% A; 26-30 min, 80% A.

1.7 STATISTICAL ANALYSES OF DATA

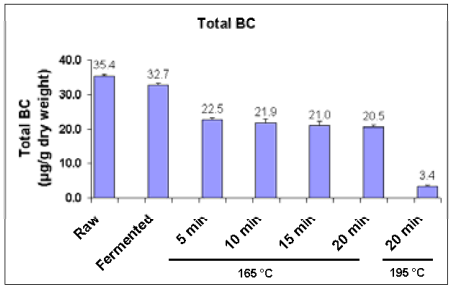
All statistical analyses were performed using SPSS (version 14.0, SPSS Inc., Chicago, IL). All data are expressed as means ± SEM and statistical significance was set at level α of $P < 0.05$. Means for all experiments were compared using one way analysis of variance (ANOVA) with Fisher's Protected LSD as a post hoc test.

RESULTS

Isomers of β-carotene in raw tuber of cassava cultivar

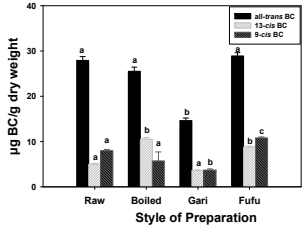


Stability of β-carotene during preparation of Gari*



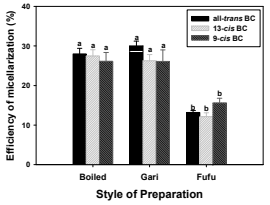
* Data are means for n = 3 replicate samples.

Quantity of BC isomers in raw and processed Cassava*



* Data are means ± SEM (n = 6).

Efficiency of micellarization of BC during simulated small intestinal digestion of fufu was approximately one half to that from boiled cassava and gari*



* Data are means ± SEM (n = 6).

Retinol Activity Equivalence (RAE) from gari and fufu is approximately one half to that of boiled cultivars of cassava on isocaloric basis

	Boiled	Gari	*Fufu*
Retention	---	↓	---
Bioaccessibility	---	---	↓
Calorie (kcal/g DM)	3.8	3.8	4.0
RAE (per 500 kcal)	96	51	48

SUMMARY

- Contrary to proposed theory, BC is relatively stable during fermentation of cassava
- Roasting during preparation of gari was associated with major loss of all-*trans* BC and concomitant increase of 13-*cis* BC
- Despite greater retention of BC during preparation of *fufu* as compared to gari, bioaccessibility of BC during simulated digestion was markedly reduced
- As RAE of *cis*-isomers of BC is ≤ 50% that of all-*trans* BC, isomeric profiles in cultivars must be screened to select those with greatest pro vitamin A activity

CONCLUSION

Processing cassava not only has impact on stability of BC but also its bioaccessibility

ACKNOWLEDGEMENTS

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